Motility Proteins and the Origin of the Nucleus

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ABSTRACT

Hypotheses on the origin of eukaryotic cells must account for the origin of the microtubular cytoskeletal structures (including the mitotic spindle, undulipodium/cilium (so-called flagellum) and other structures underlain by the 9(2)+2 microtubular axoneme) in addition to the membrane-bounded nucleus. Whereas bacteria with membrane-bounded nucleoids have been described, no precedent for mitotic, cytoskeletal, or axonemal microtubular structures are known in prokaryotes. Molecular phylogenetic analyses indicate that the cells of the earliest-branching lineages of eukaryotes contain the karyomastigont cytoskeletal system. These protist cells divide via an extranuclear spindle and a persistent nuclear membrane. We suggest that this association between the centriole/kinetosome axoneme (undulipodium) and the nucleus existed from the earliest stage of eukaryotic cell evolution. We interpret the karyomastigont to be a legacy of the symbiosis between thermoacidophilic archaebacteria and motile eubacteria from which the first eukaryote evolved. Mutually inconsistent hypotheses for the origin of the nucleus are reviewed and sequenced proteins of cell motility are discussed because of their potential value in resolving this problem. A correlation of fossil evidence with modern cell and microbiological studies leads us to the karyomastigont theory of the origin of the nucleus. Anat Rec 268: 290–301, 2002.

Key words: karyomastigont; microtubular cytoskeleton; mitosis; proterozoic cell evolution; symbiogenesis

Biologists agree that bacteria (cells of prokaryotic organization) preceded nucleated organisms (eukaryotes) in the history of life on Earth. Fossil evidence in the form of stromatolites that represent the lithified remains of microbial communities, organic molecules extracted from shales, and especially in situ microfossils preserved in cherts have produced a body of literature that attests to the prior appearance of prokaryotes. An accessible, well illustrated introduction to the early fossil record has been presented (Schopf, 1999). By the beginning of the Cambrian period of the Phanerozoic eon, 541 million years ago, a vast animal fossil biota was present on the major continents of the Earth. Direct evidence for fossil remains of eukaryotic organisms in the Archean eon is lacking. By inference, at some time between the end of the Archean eon (2,500 million years ago) and the beginning of the Cambrian, the nucleated cell evolved. Evidence for the eukaryotic cell, the morphological unit of which all protoctists, animals, fungi, and plants are composed, may even extend to the earliest Proterozoic eon (2,500–541 million years). Although the date of appearance of the earliest nucleated organism is in question the Proterozoic proliferation of an abundance of eukaryotes is indisputable. The most conspicuous and well-documented of the Proterozoic

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eukaryotes are the Ediacarans of the Vendian era (750–541 mya). At more than two dozen localities around the world, more than 20 genera and 35 species of the Ediacara biota are known. The Ediacarans were mostly psammophilic, intertidal beings. They have been extinct since the early Cambrian, and are found preserved in sandstone. Large extant protoctists (e.g., cellular slime molds, kelp, and calcified red algae) and most Ediacarans display a peculiar form of multicellularity (termed "metacellularity" by McMenamin, 1998). Ediacarans lack a head, digestive system, muscle, and other tissue. They display features that, taken together, suggest they were not animals (McMenamin, 1998). Accordingly, they are the Ediacara biota, not fauna. The fossils of the late Proterozoic (e.g., "large" microorganisms (robust acritarchs), including medusoid coelenterates, polychaete worms, and a number of others) unambiguously indicate that the nucleated cell had long since originated.

Here we constrain the problem and ask when, where, and in what populations of predecessors did the first nucleated cell evolve? We examine the transition between prokaryotes with DNA organized as nucleoids and the earliest eukaryote, i.e., any organism composed of cells with at least one membrane-bounded nucleus. We review the salient differences between these two great groups of organisms and the theories that purport to unite them via evolution.

PROKARYOTE–EUKARYOTE EVOLUTIONARY TRANSITION

The several concepts concerning the origin of nucleated organisms can be organized into two mutually exclusive groups: the nonsymbiotic (equivalent to the "direct filiation") ideas, and those based on symbiosis. The nonsymbiotic theories posit a direct origin of eukaryotes from the initial forms of life on Earth.

The "progenote" (Woese, 1998) or the "chronocyte" (Hartman and Fedorov, 2002) evolved by direct filiation from their predecessors. These authors assign a symbiotic origin to the mitochondria (from proteobacteria) and the photosynthetic plastids (from cyanobacteria). By inference, in both of these schemes the fundamental eukaryotic features of protein synthesis and cell motility evolved by direct filiation prior to any symbiotic events. The nucleus itself was incorporated into the chronocyte by symbiosis (Hartman and Federov, 2002) or symbiosis played no role in the origin of the Eukarya (Woese, 1998).

Molecular phylogenetic studies have led most authors to recognize *some* symbiogenetic event in eukaryosis, although detailed scenarios vary. The idea of "genetical annealing," for example, which postulates a hypothetical, not quite cellular, "progenote" domain, skirts the issue (Hartman and Federov, 2002). Ideas concerning filiation of eukaryotes directly from prokaryotes (such as the evolution of eukaryotes in the transition from cyanobacteria to red algae (Pickett-Heaps, 1974) or Mignot's (1996) scheme of the diversification of microtubule organizing centers in nucleated organisms nonsymbiotically from unidentified microbial ancestors) have been in the literature since the publication of a study by Church (1919). So has the concept that the nucleus itself evolved as a symbiont in host cytoplasm (Pickett-Heaps, 1974).

Variant views of the origin of the earliest nucleated organisms that involve symbiogenesis (Martin, 1999) (traceable to the turn-of-the-century work of Konstantin Mereschkovsky (1910)) have been greatly elaborated in the last two decades (Sapp, 1994). We briefly review these and mention some criteria for proof of the most adequate explanation of this cellular discontinuity. Most scientists consider it the largest gap in both living and fossil organisms. Here we refer to recent literature that presents our preferred explanation of the origin of the nucleus (Chapman et al., 2000; Margulis et al., 2000). Our view is that the nucleus emerged inside the archebacterial eubacterial chimera to join genes of these disparate partners and segregate them to offspring. The nucleus that began as a joint symbiotic organelle attached to the motile original symbiont was liberated from this early organellar system, which is known as the karyomastigont (Figs. 1 and 2). The karyomastigont was first identified and named by Janicki (1915). Nuclear liberation, by hypothesis, occurred in several to many lineages of early eukaryotes. The karyomastigont is a robust structure observed in *Chlamydomonas* studies on kinetosome-centriole DNA, and even in the fossil record (Fig. 3, Wier et al., 2002).

From the first nucleated amitochondriate cell, in the anoxic environment of the late Archean or early Proterozoic eon, the nuclear-microtubule organizing center system was related to the maintenance and distribution of genes of the symbiotic partnership, and hence to intracellular motility. The great proliferation of knowledge concerning the proteins of cell motility, including mitotic motility, convert this theoretical problem of early cell evolution into one amenable to direct experiment. A framework for verifiable consequences of reconstruction of the most likely evolutionary route from prokaryotes to the earliest eukaryotes is developed here. The karyomastigont theory of the origin of the nucleated cell is depicted here (Fig. 4) to be compared with other symbiogenetic and nonsymbiogenetic concepts.

Prokaryotes, by definition, lack nuclei. Their DNA molecule, often mistakenly called a chromosome, usually forms a circle and is generally not bound to proteins, like the histone molecules, which wrap the DNA in nucleosomes in eukaryotes. Some bacteria have DNA organized into nucleoids that are visible with electron microscopy, but these almost always lack a surrounding membrane.

In certain eubacteria ("planctomycetes" from soil and freshwater), nucleoids are bounded by a single intracytoplasmic membrane (Lindsay et al., 2001). The DNA region in *Gemmata obscuriglobus* is surrounded by two nucleoid membranes (Fuerst and Webb, 1991). The cells of this budding bacterium, *G. obscuriglobus,* with its multitrichous swarmer stage have a nucleus-like inclusion with packed ribosomes and nucleoid DNA that gives it its name (Franzmann and Skerman, 1984). *Isosphaera,* related to *Gemmata,* has only a single intracytoplasmic membrane that surrounds its nucleoid DNA. In *Pirellula,* still another relative, the membrane is called the pirellulosome. Several new isolates from soil, freshwater, and a laboratory ampicillin solution confirm that both single and double membrane-bounded nucleoids evolved in bacteria (Wang et al., 2002). They evolved in prokaryotic cells by direct filiation; they lack nuclear lamins, pore complexes, condensable chromosomes, and other features that make it clear that these intracellular organelles are bounded nucleoids, not nuclei.

No prokaryotes have been found to date that have a motile cytoskeleton. None engulf particulate food the way an amoeba or paramecium does. None move organelles,

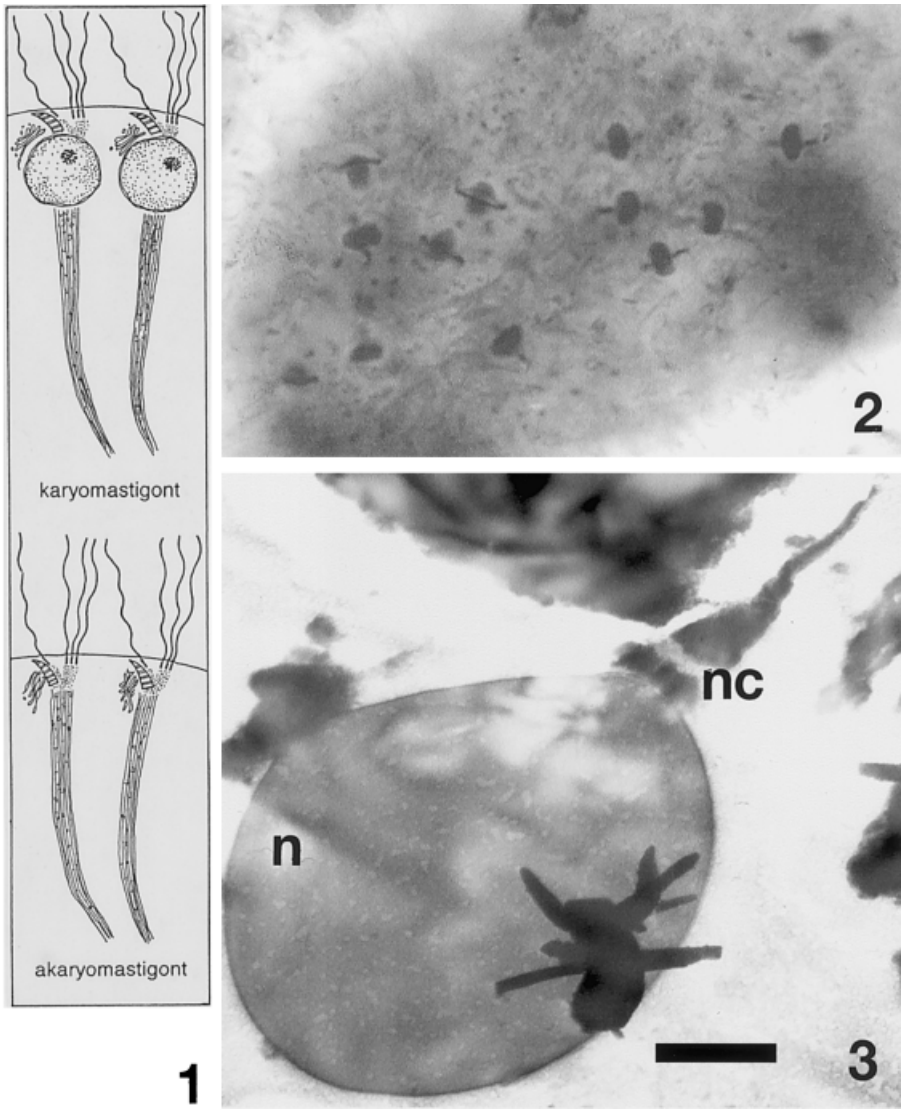

Fig. 1. The mastigont system consists minimally of a kinetosome-axoneme undulipodial complex, including the fibrous attachment to the nucleus. If the nucleus is present, the structure is the karyomastigont; if it is absent, the structure is an akaryomastigont. (Idealized drawing of these organellar systems in a trichomonad by Kathryn Delisle.)

Fig. 2. Synchronous karyokinesis in the multinucleate trichomonad *Calonympha angusta.* The pole-to-pole microtubules are seen in the extranuclear spindle.

Fig. 3. Karyomastigont preserved in 20-million-year-old amber, from the intestine of *Mastotermes electrodominicus,* a tropical termite related to the extant *M. darwiniensis.* The nucleus (n) and nuclear connector (nc) seen here in electron-microscopic thin section resemble those of the karyomastigont in *Mixotricha* today (Wier et al., 2002). Bar = 0.5 μm.

vesicles, membrane-bounded crystals, or other inclusions through the cytoplasm in the way that typifies modern eukaryotes.

Mereschkovsky (1910) hypothesized that nucleated organisms evolved symbiogenetically from a fusion of a bacterium and an ill-defined "amoeboplasm." This was in contrast to the prevalent assumption at that time, which held that nucleated organisms evolved directly from their microbial predecessors without symbiosis. The distinct characters of the modern eukaryote (membrane-bounded nuclei that divide by use of the mitotic spindle-microtubule-actin cytoskeleton) were assumed to derive directly from a single lineage of prokaryote ancestors. Today a comparable "direct filiation" view prevails which suggests that all extant eukaryotes had a mitochondriate ancestor. According to this scheme the proto-mitochondrial symbiosis in a fusion with an archaebacterium was the momentous association that led to aerobic respiration, intracellular motility, and the nuclear membrane of the eukaryote itself. We disagree. We give evidence for the idea that the earliest eukaryotes were symbiogenetically derived amitochondriate karyomastigote protists that left many descendants available for study today.

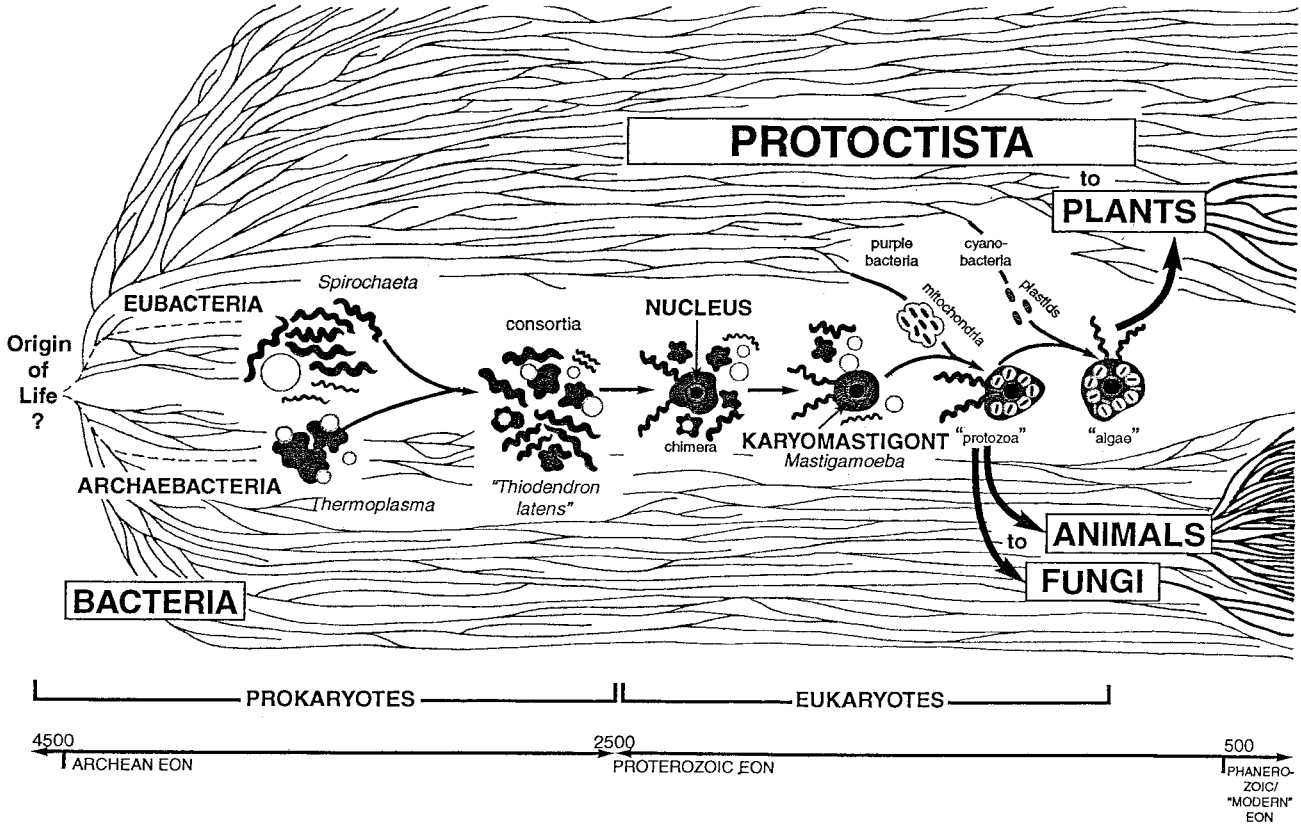

Fig. 4. Karyomastigont theory of the origin of the nucleated cell plotted against time (highly simplified). Symbiogenesis formed the archaebacterial-eubacterial chimera, in which the nucleus originated by release from its karyomastigont (left) to the present. See frontispiece, figure FM1, page ix, in Margulis and Sagan (2002). (Diagram by Kathryn Delisle.)

We of course recognize the presence of proteobacterial genes in nuclei, for example even those of the amitochondriate *Giardia;* however, we account for them in other ways. The phenomenon of bacterial endosymbiosis is so prevalent in anoxic environments that multiple bacterial symbioses cannot be precluded. Furthermore, since the eubacterial-archaebacterial association (which evolved the membrane-bounded nucleus connected as part of the karyomastigont, in our reckoning) preceded mitochondria, the eubacterial partner probably contributed nuclear genes. The question of the origin of the microtubular system still applies whether or not the earliest eukaryote was mitochondriate.

ORIGIN OF THE MITOTIC-INTERPHASE CYTOSKELETON

We disagree that microtubular structures (the mitotic spindle, undulipodia, and their associated motility proteins) evolved endogenously (without symbiosis) within the eukaryotic lineage. Most scenarios are inconsistent with evolutionary principles: nuclei and cytoskeleton evolve because of selection pressures for their usefulness! Yeasts and red algae, which lack undulipodia and have an intranuclear microtubule organizing center, represent the ancestral state for Pickett-Heaps (1974) and Mignot (1996), both of whom envisage that undulipodial motility comes after mitosis. The peduncle hypothesis, which proposes that the undulipodium evolved from an immotile stalk (Rizzotti, 2000), provides a variation and extension of this endogenous evolution. Selection pressure from the search for food led the heterotrophic protists to develop a motile stalk, on which continued pressure formed a swimming appendage that became the undulipodium. The relationship between the nucleus and the microtubule-based mastigont structure (cilium or undulipodium) is ignored in these previous works.

These nonsymbiotic hypotheses require that all eubacterial genes in the nucleus derive from the bacteria that gave rise to mitochondria and chloroplasts. They, furthermore, infer the existence of a lineage of bacteria, the pre-mitochondrial proto-eukaryotes, for which there is no evidence. Selection pressures that led the distinctive eukaryotic motility characteristics to evolve after formation of nuclei are not identified in these scenarios.

Mitochondria and photosynthetic plastids have been established to be symbiogenetic in origin. Analogously, what criteria must we apply to test the symbiotic vs. direct filiation origin of the basal eukaryote prior to the acquisition of these eubacterial organelles? First, the nuclear genome must contain traces of eubacterial genes that came from neither mitochondrial nor plastid ancestors.

Any case of acquisition of early cell motility genes or structures (such as microtubules and their associated proteins) must be traceable to the original eubacterial or archaebacterial ancestor, horizontal gene transfer from an identifiable prokaryote, or recombinant proteins emergent from the original archaebacterial-eubacterial fusion. A set of genes or a remnant genome from this putative earliest motile eubacterial symbiont should more closely resemble an extant motile prokaryote than it does any arbitrarily-chosen bacterium.

The idea of a third primordial lineage, Hartman and Federov's postulated chronocyte (2002), is nearly impossible to test because this domain of life does not now exist anywhere on Earth. Furthermore, when Hartman and Federov claim that the presence of a cell wall and lack of phagocytosis preclude the fusion of prokaryotes as endosymbionts, they display ignorance of an entire literature in which prokaryotes have been shown to live and grow in stable endocellular associations (Guerrero, 1991; von Dohlen et al., 2001). The invasion through the membrane of prokaryotes by different prokaryotes (even in the presence of a cell wall) to form stable intracellular associations did not require phagocytosis, which we agree did follow the origin of eukaryotes. We suggest that the "whole set of new cellular structures (i.e., endoplasmic reticulum. . .)" (Hartman and Federov, 2002, p. 1420) derive from eubacterial-archaebacterial integration. In this reckoning the endoplasmic reticulum is of archaebacterial origin and fused to the Golgi, which evolved from the eubacterial ancestor. This is consistent with the finding that the *N*-linked protein glycosylation pathway of the endoplasmic reticulum resembles in detail that of precursors to wall biosynthesis in archaebacteria. Studies of the archaebacterial origin of the ER membrane system were reviewed by Helenius and Aebi (2001). The Golgi membrane appears to have an entirely different evolutionary origin (Helenius and Aebi, 2001), perhaps eubacterial. Biochemical details in reports such as these are required to distinguish the conflicting concepts regarding nuclear origin.

According to the principle of evolutionary continuity, even the transition between the non-nucleated prokaryotes and the nucleated eukaryotes with extensive intracellular motile systems should have left some extant descendants. Certain anaerobic archaeprotists that dwell in anoxic environments, all of which lack mitochondria, provide us with the best clues to the origin of eukaryotes.

AMITOCHONDRIATE PROTISTS AND THE LIBERATION OF THE NUCLEUS FROM THE KARYOMASTIGONT

The Archaeprotists (anaerobic, amitochondriate protists that dwell in anoxic environments) provide the best clues to eukaryosis. Placed as the earliest-branching lineages on phylogenetic trees of eukaryotes, they comprise the archamoebae (*Mastigamoeba, Pelomyxa*), the metamonads (including the diplomonads (*Giardia*)), the retortamonads (*Retortamonas*), and the parabasalia (trichomonads and hypermastigotes). These protist cells contain a conspicuous organellar system called the karyomastigont, which is central to our evolutionary argument. In the karyomastigont a single nucleus connects to from one to four undulipodia. In the case of the many species of parabasalids, the nucleus-undulipodia system is attached also to the parabasal bodies (the Golgi complex). The confluence of molecular phylogenetic and morphological data suggest that these extant karyomastigont-bearing lineages most closely resemble the earliest eukaryotes. Other eukaryotic lineages in which the nucleus is not attached to the mastigont system (undulipodia) represent the later-to-evolve (i.e., the derived) state (Chapman et al., 2000).

An evolutionary explanation of nuclear origin must also account for the origin of nuclear division, as mitosis vastly differs from the "binary fission" of prokaryotes. The archaeprotists are characterized by a closed mitosis in which the chromosomes become attached to the persistent nuclear envelope such that their kinetochores are imbedded in the envelope. An extranuclear spindle forms with two poles, each of which is associated with a half of the mastigont. The thin mitotic spindle, called the paradesmose in some late 19th and early 20th century literature (Raikov, 1982), serves to separate both the nuclei and the undulipodia. Thus, from the earliest times reproduction of the mastigont occurred in a semi-conservative fashion: each offspring cell received old and new kinetosomes, and neither cell had to produce kinetosomes de novo (in association with an existing kinetosome).

The archamoebae, some of which have one undulipodium per karyomastigont, are not explained by this model. Little is known about their karyokinesis or sexuality. Indeed, recent molecular data do not support the archamoebae as an early branching lineage (Bapteste et al., 2002).

Some archaeprotists (diplomonads, trichomonads) are said to have evolved from mitochondria-containing cells because their nuclei contain gene sequences similar to genes in mitochondria. Similarly, the hydrogenosome found in trichomonads is said to be from the same symbiosis that led to mitochondria (Martin and Müller, 1998). Other archaeprotists, for which biochemical evidence is not available, are thought to be related to mitochondriate forms by the presence of cytoplasmic electron-dense membrane-bounded structures, as in the pelobionts (Walker et al., 2001), or because they are related to other organisms with membrane-bounded bodies, such as oxymonads (Dacks et al., 2001). However, the presence of nonmitochondrial endosymbiotic eubacteria or their remnant gene sequences have not been excluded. Many, if not all, of archaeprotists studied by electron microscopy harbor prokaryotic endosymbionts, often several types simultaneously (Daniels and Breyer, 1967; Dolan, 2001). We suggest that only one or very few eubacteria-protist symbioses led to the mitochondria of the protist lineages from which animals, fungi, and plants evolved. The vast majority left obscure, peculiar descendants—protoctists that today live in anoxic or microxic (dysaerobic) habitats.

MOTILITY PROTEINS AND THE SEARCH FOR BACTERIAL HOMOLOGUES

The question of the evolutionary origin of the nucleus is inseparable from consideration of the mitotic and microtubule motility systems. Final resolution of this problem requires an understanding of the origin, evolution, and selective advantages in natural populations of the complex set of motility proteins that abound in eukaryotes. Here we define "motility protein" as a polypeptide found in nature (that is, in an in vitro assay usually with other necessarily associated proteins) capable of movement. Protein motility can be visualized at the level of the light micro-

TABLE 1. Sequenced mitotic and motility proteins

Protein	Approximate mw	Ion/nucleotide	Significance
Astrin	134 kDa	–	Specific association with spindles, may play a role in spindle structure
BUB (budding uninhibited by benzimidazole)	140–150 kDa	Activates GTP-ase	Mitotic checkpoint component, kinetochore tension-sensitive
Cenexin	96 kDa	–	Acquired by immature centriole at the G2 to mitosis transition, inner centriole wall
CENP-A	17 kDa	–	Involved in mitotic kinetochore assembly or mutation
CENP-E	312 kDa	GTP	Assists in kinetochore microtubule binding, maintains spindle pole structure, kinesin-like
Centrin (cyclin-dependent kinase)	20 kDa	Ca++, none	Anti-spasmin antibodies recognize it, localizes to centrosome, MT organization
Dynein (ATPase)-end directed	>1000 kDa dogbone structure	ATP ADP	Motor protein
Dynactin	Varies	None	Binds membrane, binds NuMa, binds kinetochore
Kinesin (ATPase) + or – end directed	120 kDa (varies)	ATP ADP	Motor protein
Kinectin	160 kDa	GTP	Binds membrane, moves endosomes
MAD (mitotic arrest deficiency)	25 kDa	?	Mitotic checkpoint component kinetochore is tension sensitive, tethers microtubules to poles
NuMA	240 kDa	GTP	Spindle attachment, polar, dogbone-shaped
Pericentrin	220 kDa	None	Lattice in centriole rings of gamma tubulin, it is the major PCM component, centrosome and mitotic spindle formation and function
Tubulin			
Alpha tubulin	50 kDa	CA++, GTP	α-β heterodimers form walls of microtubules
Beta tubulin	50 kDa	CA++, GTP ~GDP	
Gamma tubulin	50 kDa	–	Defines microtubule polarity nucleating agent for centriolar replication
Delta tubulin	51 kDa	–	Forms triplet microtubules of centrioles and kinetosomes

– = not known.

scope, generally in combination with the appropriate acidity/alkalinity (hydrogen ion and/or hydroxyl ion concentrations), concentrations of small molecules (GTP or ATP) and ions (potassium, sodium, or calcium salts), and conditions (temperature, hydration, pressure, tension, etc.). The transport of chromosomes in mitosis by use of kinetochore motor proteins, separation of nuclei after karyokinesis or of offspring cell cytoplasm after cytokinesis, movement of vesicles and granules along cytoskeletal microtubules, transport of mitochondria and food particles along axopods, locomotion of cells by undulipodia due to axonemal undulation, and many other processes of clear selective advantage in eukaryotes require motility proteins. The goal is to relate the domains of motility proteins to their evolutionary predecessors.

Very few protein homologies between *Treponema pallidum* and *Borrelia burgdorferi* were discovered by revelation of the primary sequence of their genomic DNA (Fraser et al., 1997, 1998). However, FtsZ, the homologue of tubulin involved in prokaryotic cell division, appears to be universal in eubacteria and archaebacteria (Erickson and Stoffler, 1997). A protein called MreB, which is directly involved in bacterial cell division, apparently is a homologue of actin (a major component of the cytoskeleton and a ubiquitous eukaryotic motility protein (van den Ent et al., 2001)). These observations are useful to test our hypothesis for the origin of the nucleus and its associated microtubule cytoskeleton (Chapman et al., 2000; Margulis et al., 2000). Unlike any others, our concept posits that certain eukaryotic motility protein active sites (relevant domains) will be more homologous to cytoplasmic (protoplasmic cylinder) proteins in spirochetes than they will be to any arbitrarily chosen prokaryotic proteins (for example, those from *Escherichia coli* or the cyanobacterium *Synechococcus* (Bermudes et al., 1987)). We briefly list potential candidate proteins whose domains may have retained motility functions homologous to those in spirochete cytoplasm (listed in Table 1).

Some pillotinaceous spirochetes bear cytoplasmic tubules in their protoplasm (Bermudes et al., 1994); even a "cytoplasmic tubule-associated center" morphologically similar to "microtubule-organizing centers" of fungal nuclei was reported in termite spirochetes (Wier et al., 2000). These tubules resemble microtubules, but no biochemical data accompany the electron micrographic images. Certainly, however, if spirochetes are ancestral to eukaryotic motility systems as postulated, their highly conserved functional protein domains will be detected. Candidate proteins for homology studies include 50 kDa proteins α- and β-tubulin, which comprise the heterodimers of microtubules, and all others listed in Table 1. Studies might seek, for example, homologies with γ-tubulin, which forms rings 25 nm in diameter that are capable of nucleating microtubule assembly, possibly by the transient stabilization of the minus-end (Moritz et al., 1995). This protein,

which is sensitive to the mitotic stage, increases in concentration during prophase (Khodjakov and Rieder, 1999). Other tubulins (δ-, ε-, ξ-, and η-tubulins) are involved in the formation of centriole microtubules, but unlike α-, β-, and γ-tubulins are not ubiquitous in eukaryotic organisms (reviewed by Dutcher, 2001). Thus they are less likely candidates for homology studies.

Pericentrin nucleates microtubules in some eukaryotic cells (Zimmerman and Doxsey, 2000). This 220 kDa protein present at the centrosomes throughout the cell cycle colocalizes with γ-tubulin (Khodjakov and Rieder, 1999; Doxsey et al., 1994). Both pericentrin and γ-tubulin are cargo of dynein ATPase, the huge (MW > 1 megadalton) minus-end directed motor protein (Young et al., 2000). Dynein possesses a symmetric dog-bone structure with a particle- and membrane-binding light chain and a force-generating heavy chain. The latter binds tubulin. Dynein, which composes the "arms" of each of the nine doublets of microtubules of the undulipodium, is responsible for their beating motion. Dynein is attached to the spindle and is also found at chromosome kinetochores after the nuclear envelope breaks down. Dynein binds to both spindle microtubules and the poles (Echeverri et al., 1996; Wordeman et al., 1996). Dynein, in live cells as an ATPase, always binds to other proteins, including dynactin (Echeverri et al., 1996). Dynactin, a connector protein, binds the nuclear mitotic apparatus (NuMA) protein (reviewed by Compton, 1998; Zimmerman and Doxsey, 2000). Both dynein and dynactin are involved in mitotic spindle organization (Gaglio et al., 1997). NuMA, a 240 kDa dumbbell-shaped protein located during interphase inside the nucleus, where it is considered to be a structural component, is transported to the poles during cell division, where it appears to tether microtubules to the poles (reviewed by Compton, 1998).

Analogous to dynactin is the connector protein kinectin, which binds the light chain of the motor protein kinesin to membranes (Kumar et al., 1998). Kinesin, a 120 kDa ATPase that is either plus- or minus-end directed, transports particles (i.e., vesicles, lysosomes, and secretory granules) along microtubules. Mitotic centromere-associated kinesin is involved in chromosome segregation during anaphase (Maney et al., 1998). Multiple kinesins are found in undulipodia. Two are tightly associated with the microtubular central pair apparatus of motile axonemes. The function of the central pair kinesins is unknown; they may contribute to the known rotation of the central pair apparatus. A third kinesin (kinesin-II), found in both motile 9(2)+2 axonemes and nonmotile 9(2) + 0 sensory cilia, is associated with a transport process that was first identified in *Chlamydomonas* (Cole et al., 1998).

The microtubule-dependent centromeric motor protein E (CENP-E) is related to kinesin but requires GTP, not ATP, to effect movement. CENP-E is located at the kinetochores of the chromosomes until mid-anaphase, when it migrates to the midzone of the spindle. Despite the importance of the trilaminar kinetochore (which directs the segregation of chromosomes in mitosis and meiosis), its molecular architecture remains poorly understood. The best known component of the kinetochore plates is CENP-C, a protein that is required for kinetochore assembly and is thought to be involved in kinetochore size determination. The 17 kDa CENP-A protein is concentrated in active centromeres in the region of the inner kinetochore plate. It copurifies with nucleosomes, and recruits and assembles other kinetochore proteins, including CENP-C (Van Hooser et al., 2001). A specific nucleosomal substructure for the kinetochore was hypothesized by Warburton and colleagues (1997).

An unexpected connection between structural components of the nuclear pore complexes and the chromosomal kinetochores has been revealed: throughout mitosis, a fraction of the nuclear pore proteins localizes to the kinetochores (Belgareh et al., 2001). The mitotic cytoplasm contains kinetochore-binding competent histone nuclear pore CENP-A proteins. They dynamically interact with the kinetochores. These kinetochore proteins locate in the heterochromatin typical of the centromeric regions of mammalian and other chromosomes.

Bub1 (budding uninhibited by benzimadazole), found at kinetochores of aligned chromosomes, is essential for cell cycle arrest in response to spindle damage or chromosome misattachment (Taylor and McKeon, 1997; Sharp-Baker and Chen, 2001). Bub proteins are necessary for the arrest of cell cycle progression upon the loss of microtubule function (Hoyt et al., 1991). Bub1 and bubR1 are tension-sensitive proteins that bind to kinetochores in the absence of sufficient spindle tension, and generate a mitotic checkpoint response that delays anaphase until all chromosomes are attached (Skoufias et al., 2001).

Mad (mitotic arrest deficiency protein) is a small protein (25 kDa) that is involved in feedback control over exit from mitosis (Li and Murray, 1991). Mad2 is sensitive to microtubule attachment to the kinetochore, but not to spindle tension (Skoufias et al., 2001). Mad2, which localizes at the unattached kinetochores, is absent from kinetochores that are attached to the spindle (Chen et al., 1996). Association of mad2 with the kinetochore inhibits anaphase by preventing proteolysis of cyclin B (Li and Benezra, 1996). Mad1 responds to spindle damage, arrests cell cycle progression, and binds mad2 if spindle assembly is disrupted (Hardwick and Murray, 1995). The cell cycle is further controlled by the centrin (cyclin-dependent kinase) family of proteins (reviewed by Nagl, 1995). These small calcium-binding proteins, which are plentiful in animal cells during G1, underlie rapid contraction in certain protists. Another member of the family that is important in regulating the cell cycle, cdc2, is localized to the centrosome throughout the cell cycle (Pockwinse et al., 1997).

Another conserved protein that merits further study is cenexin, which apparently is acquired by young centrioles during the transition from G2 to mitosis. Cenexin, as diagrammed in Chapman et al. (2000), forms the inner centriole wall at the distal end. Its acquisition is claimed to enable the centriole to become a kinetosome (Lange and Gull, 1995). Astrin, too, is a structural protein specific to the spindle, as its name implies. It associates with kinetochores on only those chromosomes that are properly aligned at the metaphase plate (Mack and Compton, 2001).

The spatial distribution of some of these proteins in the animal cell in mitosis is diagrammed in Figure 5. Depicted at higher magnification are the cytoplasmic proteins associated with a single microtubule (Fig. 6). The specific eukaryotic motility proteins listed and shown provide the best candidates in the search for prokaryotic, specifically spirochete, evolutionary homology.

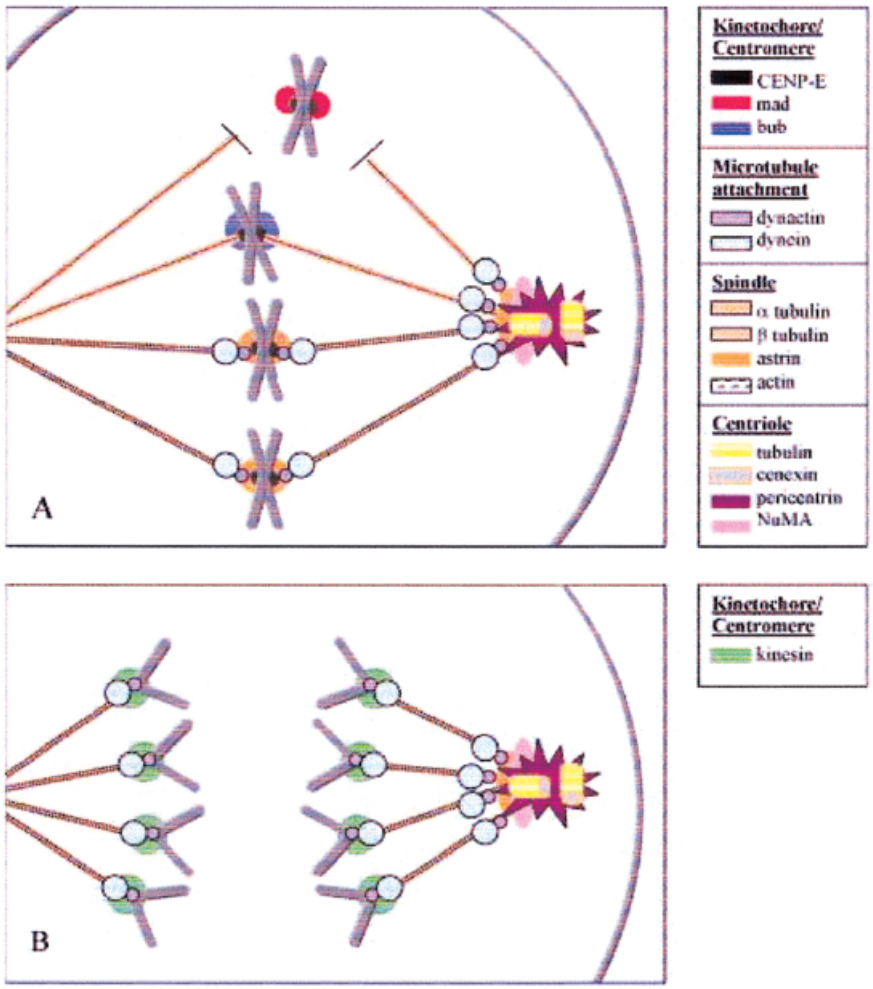

Fig. 5. Motility protein locations in mitotic cells. Structures and motility proteins are depicted with respect to general location and are not drawn to scale. **A:** Prometaphase/metaphase cell. CENP-E, which is required for spindle attachment, localizes to the outer kinetochore until mid-anaphase. Mad2 localizes to those kinetochores that are not attached to microtubules during anaphase. If spindle damage occurs, Mad1 undergoes phosphorylation and binds mad2 where the microtubule connections have been severed. Bub1 undergoes autophosphorylation, binds Bub3, and coats kinetochores that have microtubules attached (but only if tension is lacking). Bub3, which is necessary for kinetochore-microtubule attachment, associates with CENP-E. Dynactin binds to the much larger dynein ATPase. This complex of proteins binds microtubules at both the kinetochores and centrioles. The alpha and beta tubulin dimer is the major constituent of the microtubules. Astrin localizes throughout the spindle and concentrates at the poles and at the kinetochores of aligned metaphase chromosomes. Actin is another structural component of the spindle. The centrioles are comprised of tubulin. Cenexin is a structural component of the distal end of the inner centriole wall. Pericentrin nucleates microtubules, co-localizes at the centrosomes, and is present throughout the cell cycle. NuMA, which is located in the nucleus during interphase, is transported to the poles and attaches along the spindle during cell division. **B:** Anaphase cell. Kinesin associates with centromeres, and is involved in chromosome segregation during anaphase.

CONCLUSIONS

Any proper hypothesis of the origin of the nucleus must find evolutionary precedents for the mitotic motility system as well. The most promising approach is a specific comparison of highly conserved functional sequences of eukaryotic proteins with those of prokaryotic proteins. The karyomastigont theory of the origin of the nucleus requires specific comparison of spirochete with eukaryotic motility proteins. The protein domains with the highest priority might be those required for locomotion, tension sensitivity, nuclear membrane-chromosomal (including kinetochoric) DNA association, and especially the 600+ proteins that account for remarkable conservation of the centriole/kinetosome in motile protoctists, plant sperm, animal sensory cilia, etc. We predict greater homology of eukaryotic motile proteins with functionally comparable proteins of carefully chosen extant spirochetes than with other bacteria. Hartman and Federov (2002) predict no homology of these quintessentially eukaryotic proteins with extant prokaryotes. Martin and Müller (1998) would seek homology amongst methanogens. By contrast, Gupta (1998) posits a unique symbiogenetic origin of eukaryotes from an archaebacterial-eubacterial chimera. Gupta's candidate for the eubacterial origin of these eukaryotic

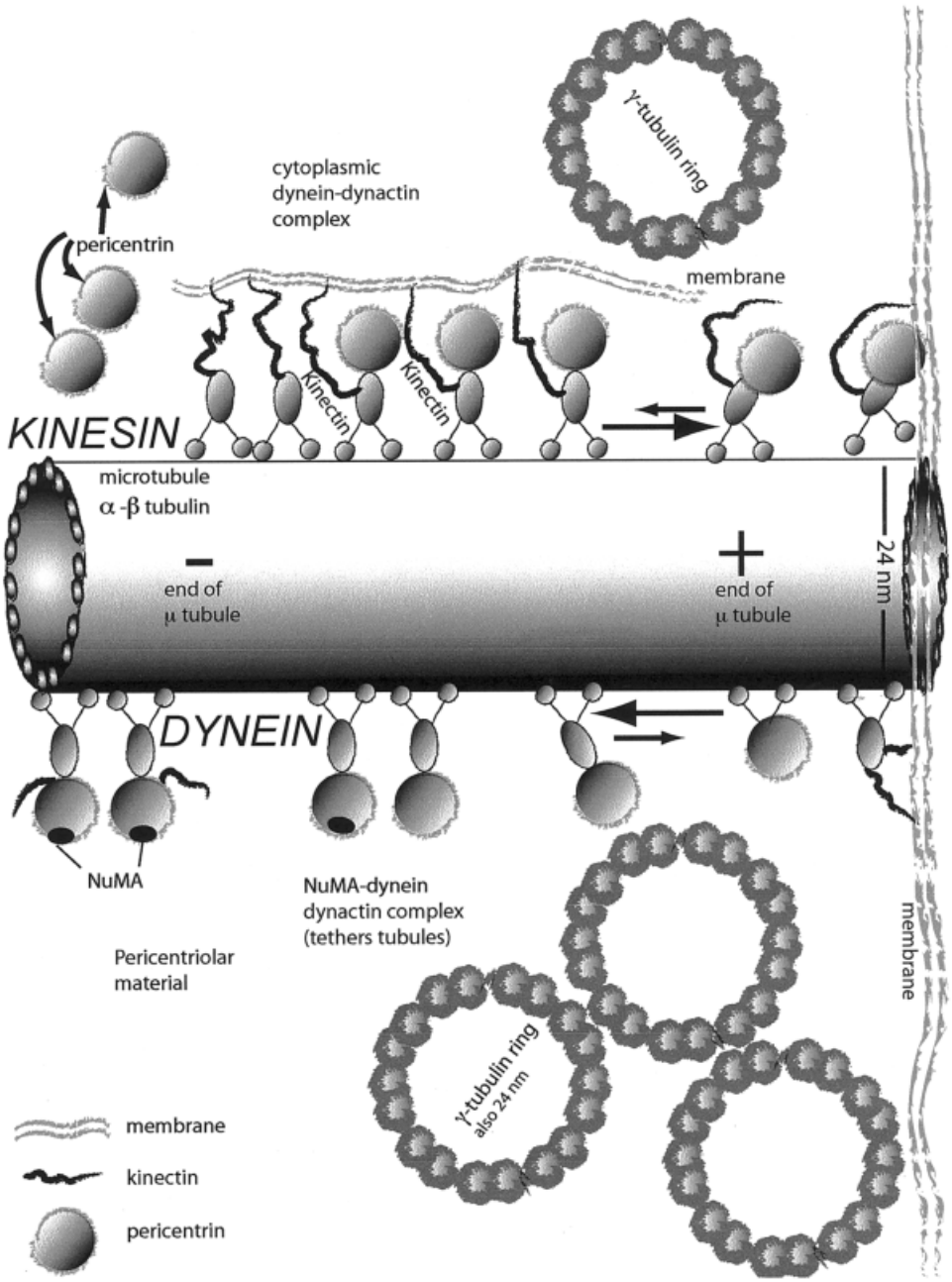

Fig. 6. Cytoplasmic microtubule-associated motility proteins in eukaryotic cells. Candidates for future study of protein homologies include the pericentrin, dynactin, kinectin, NuMA, and γ-tubulin. (Drawing by Kathryn Delisle based on information from Stephen Doxsey and from the literature.)

proteins is *Chlorobium,* or some other photosynthetic bacterium. He rejects our idea that his chimera's eubacterium is related to spirochetes.

Martin and Müller's (1998) hydrogen hypothesis states that mitochondria and hydrogenosomes share a common prokaryotic ancestor. The symbiotic relationship between an anaerobic, hydrogen-dependent, autotrophic archaebacterium (a methanogen) and a respiring, anaerobic, heterotrophic eubacterium that gave off hydrogen as a waste product (a fermenter) began as a syntrophy and, by their reckoning, became the first eukaryote (Martin and Müller, 1998). The mitochondrial symbiont that began as an α-proteobacterium was taken up by an hydrogen-dependent archaebacterium, most likely a methanogen. The symbiotic association between these two types of prokaryotes developed in an anoxic environment replete with carbon dioxide and hydrogen. Upon removal from this environment, the archaebacterium became fully dependent on the hydrogen/carbon dioxide-producing eubacterium. Selection led to a larger surface area that more

easily surrounded the eubacterial symbionts. Use of the carbohydrate metabolism and membrane transport systems of the eubacterium led to a loss of autotrophy, which resulted in the evolution of a heterotroph as the α-proteoeubacterium became the ancestral mitochondrion. The evolution of aerobic mitochondria followed. All extant amitochondriate protists evolved by the secondary loss of mitochondria (oxygen-respiring eubacterial symbionts or related relict organelles, the hydrogenosomes (Martin and Müller, 1998)).

This hypothesis fails to account for the origin of the central, defining eukaryotic organelle: the mitotic nucleus. While Martin (1999) dismisses an endosymbiotic origin of the nucleus and ignores the motility proteins, he posits that expression of eubacterial genes for lipids produced the nuclear membrane and the endoplasmic reticulum.

A similar syntrophy hypothesis, proposed by López-Garcia and Moreira (1999) differs from Martin's (1999) in that it involves symbiotic associations between a methanogenic archaebacterium and two different types of eubacteria. They argue that, initially, a symbiotic association existed between a δ-proteobacterium, which reduced sulfate to produce hydrogen and carbon dioxide. A methanogen consumed these waste products. A symbiotic association developed with a methanotrophic α-proteobacterium at the same time or shortly thereafter. That myxobacterial proteins are homologous to those in the eukaryotic signaling pathways, and that methanogens possess lipids and pathways to produce them that are homologous to those of eukaryotes are offered in support of this idea. A protonuclear region with the cytoplasm and membranous structures of the archaebacterium is postulated to have arisen in this association. Methanogens, presumably with DNA-associated enzymes like those of eukaryotes, contain histone protein organized into small nucleosome-like structures. The eubacterial genes were transferred to the genome of the archaebacterium, where genes for metabolism replaced those of the methanogen, and genes encoding the genetic machinery of the eubacteria were lost (López-Garcia and Moreira, 1999).

These syntrophy hypotheses are supported by observations of myriad symbiotic associations between methanogens and proteobacteria in nature. One might expect evidence of multiple independent origins and evolution of eukaryotic cells, as Gupta (1998) noted. However, Gupta drew upon molecular sequence data to stress that all eukaryotes stem from a common ancestor. With these scenarios one might expect evidence for remnants of methanogenesis in extant eukaryotes, or for eukaryosis in methane-rich environments. None has been proffered.

An alternative hypothesis for eukaryote origins, the chimera, was detailed by Gupta (1998). Through analysis of molecular sequence data based on complete protein sequences (as opposed to complete protein sequences inferences limited to ribosomal gene data). Gupta concluded that thermoacidophilic archaebacteria (eocytes) are the closest relatives to eukaryotic cytoplasm. He hypothesized that an initial merger of the archaebacterium by an α- or δ-proteobacterium led to formation of the first eukaryote when the archaebacterium topologically lies within the eubacterium. Full integration of the two genomes then occurred (Gupta, 1998; Gupta and Golding, 1996). Subsequent endosymbioses by the nucleated microorganism that acquired proteobacteria gave rise to mitochondria and plastids. Gupta failed to explain the biological context of a fusion between such distantly related prokaryotes, and offered no scenario for the origin of the mitotic spindle or other motility features (as Martin and Müller (1998) pointed out).

We favor hypotheses of syntrophy, especially in the integration of Gupta's (1998) archaebacterial-eubacterial chimera, as the selection pressure that formed the initial karyomastigont-bearing protist. However, we suggest that Searcy's (2000) concept is the most stimulating to research. His idea, incorporated into our karyomastigont theory (Fig. 4), requires sulfur syntrophy. A thermoacidophilic archaebacterial component maintained reduced conditions for its eubacterial symbiotic partner; the latter (the motile eubacterium perhaps a *Spirochaeta*) reoxidized sulfide at least to elemental sulfur, which then served as a terminal electron acceptor for the *Thermoplasma*-like archaebacterium. Searcy's concept provides the basis for our working hypothesis (Fig. 4).

Although progress has been made whereby the various mutually exclusive ideas are now distinguishable and the tools for solution exist, we must conclude that the problem of the origin of the nucleus remains unsolved. Many avenues of investigation are relevant: natural history, microbial community analysis (especially of anoxic habitats), paleobiology (especially pre-Phanerozoic micropaleontology), protistology, genomics, proteomics, and aspects of cell biology (such as immunofluorescence studies of motile and tension sensitive proteins). Communication between scientists trained in traditionally isolated lines of investigation is desperately needed to reconstruct the jump across the greatest gap in in the history of life on Earth: the evolutionary transition from prokaryote to eukaryote.

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